The Molecular-Cellular-Field Continuum of Mercury Detoxification

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Stewardship requires controlling bioavailability $MeHg^+$ and Hg^{2+}, the substrate for methylation. Effective Hg detoxifying proteins have evolved in many bacteria and archaea. Enhancing removal or sequestration of Hg^{2+}/$MeHg^+$ by naturally Hg resistant (HgR) bacteria means knowing how these proteins work. We study HgR genes of γ-proteobacteria and actinobacteria abundant in high Hg areas of the ORR and report here on the mechanisms of the *mer* reductase and demethylase and regulatory proteins that optimize their expression to rapidly and completely convert Hg^{2+} or RHg^+ to volatile, poorly biomagnified Hg^0. **ACTINOBACTERIAL MerA/MerB**: Relatives of *Streptomyces lividans* are found in high Hg regions of the East Fork Poplar Creek of the ORR. We study their co-evolved MerA/MerB proteins. *S. lividans* MerA (SLMerA) lacks the tethered NmerA domain found in γ-proteobacterial MerA, and its MerB (SLMerB) lacks a cysteine of proteobacterial MerB essential for Hg^{2+} transfer to NmerA, but has a distinct C-terminal cysteine pair. To examine the role of this cysteine pair in RHg^+ binding and Hg^{2+} release, we generated an active site mutant with only the C-terminal cysteine pair (SSCCSLMerB) and a mutant with only the active site cysteines (CCAASLMerB). Results with wild type SLMerB and the mutants will be presented. **Hg ISOTOPE FRACTIONATION BY PURIFIED MerA**: Distinct signatures in Hg isotope fractionation for different chemical processes are important for distinguishing biotic and abiotic contributions to natural Hg cycling. Kritee et al (2007) measured Hg isotope fractionation by intact cells expressing *mer* genes and proposed that MerA is largely responsible for the observed effects. To test this in vitro, we're measuring Hg isotope fractionation with a purified γ-proteobacterial MerA. Experimental design and progress will be presented. **PROTEOBACTERIAL REGULATION OF *mer* EXPRESSION**: In γ-proteobacteria, repressor-activator MerR holds RNA polymerase (RNAP) at the *mer* operator-promoter (MerOP) until Hg^{2+} provokes it to let RNAP transcribe. MerR binds MerOP and Hg^{2+} tightly, so anti-activator, MerD, must stop expression when all Hg^{2+} is reduced. We've made MerD monoclonal antibodies for in vitro interaction studies with MerR, MerOP and RNAP. In vivo RT-qPCR shows *mer* mRNA declines once Hg^{2+} is reduced if *merD* is wildtype and are currently testing *merD* mutants. We also find Hg^{2+} binds a 38 bp MerOP DNA in the absence of thiols (as in acute Hg^{2+} exposure) at 3 distinct, high affinity sites (K_{form} ~10^{20} M^{-1}). EXAFS shows expected nitrogen ligandsbut TA:AT slippage, the believed basis of Hg^{2+} binding in artificial systems, cannot occur in MerOP. Free bases don't bind Hg^{2+}, so DNA's high affinity must involve base-pairing or -stacking, an idea we are testing. We also find *E.coli* cells bind >3-fold more Hg^{2+} via N or O ligands than their available thiols. So, cellular DNA may be a large sink of Hg^{2+} with unknown turnover rate. **ACTINOBACTERIAL REGULATION OF *mer* EXPRESSION**: Actinobacteria control the *mer* operon with a simple ArsR-type repressor. *S. lividans* MerR (SLMerR) is a distinct clade of the ArsR family. Homology models based on ArsR regulators with 3D structures show a candidate Hg^{2+} binding site in SLMerR's DNA binding helix, a radical departure from metal sites in other ArsR regulators. SLMerR over-expression proved toxic in *E.coli* which lacks the tRNA profile to translate actinobacterial mRNA, resulting in ribosome malfunction. With help from the UT-ORNL group we synthesized an *S.lividans merR* gene optimized for expression in *E.coli* and anticipate good production of this unusual ArsR-type regulator.